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Original Article

Synthesis of Antifungal 5-Anilino-1,2,4-oxadiazoles and 5-Anilino-1,2,4-thiadiazoles, and Their Inhibitory Effects on Fungal Sterol Biosynthesis

Izumi KUMITA* and Atsushi NIWA#

Odawara Research Center, Nippon Soda Co., Ltd., Takada, Odawara 250-0216, Japan

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In the screening process we found that two 1,2,4-oxadiazole compounds showed antifungal activity against *Trychophyton mentagrophytes*. Microscopic observation revealed that these compounds inhibited mycelial growth with morphological changes without inhibition of germination. When treated with the oxadiazoles, ergosterol of *Candida albicans* decreased strikingly and much squalene was accumulated. These results firmly suggested that the compounds were new squalene epoxidase inhibitors. We synthesized the related oxadiazoles and thiadioazoles having various substituents to investigate the structure-activity relationships. Among the synthesized compounds, 3-(2,6-dichlorophenyl)-5-(4-chloroanilino)-1,2,4-thiadiazole (**51**) showed the highest fungicidal activity against *Botrytis cinerea*, and accumulated squalene remarkably. This compound is of interest as a new lead of squalene epoxidase inhibitors.

Key words: squalene epoxidase inhibitor, antifungal activity, *Trychophyton mentagrophytes, Botrytis cinerea*, 3-phenyl-5-anilino-1,2,4-oxadiazoles, 3-phenyl-5-anilino-1,2,4-thiadiazoles.

INTRODUCTION

Although many chemicals which inhibit the biosynthesis of ergosterol from lanosterol are known as antifungal compounds in medicinal and agrochemical fields,[1,2] there are a small number of congeners which inhibit a pathway before lanosterol in sterol biosynthesis. We were interested in the squalene epoxidase as a target of sterol biosynthesis inhibitors, and attempted to discover a new class of fungicides. In the course of screening projects for the novel fungicides, we found some substituted 5-anilino-3-phenyl and 3-benzyl 1,2,4-oxadiazoles showed antifungal activity *in vitro* against dermatophyte fungi, *Trychophyton mentagrophytes*. Under the microscopical observation, the characteristic effects of the compounds to *T. mentagrophytes* were similar to those of ergosterol biosynthesis inhibitors. Treatment of the fungus with these compounds also increased the accumulation of squalene in *Candida albicans*. These data suggested that the oxadiazoles might be a new type of squalene epoxidase inhibitors.

The present paper describes antifungal potencies and squalene epoxidase inhibition activity of the oxadiazoles and related compounds to understand essential features in the structure-activity relationships.

MATERIALS AND METHODS

1. Synthesis of Compounds

The oxadiazole compounds were prepared according to the established procedures outlined in Fig. 1. Most of the oxadiazoles were prepared by condensation of amidoximes (**I**) with imidocarbonylchlorides (**II**) in the presence of 1,4-diazabicyclo[5.4.0]undec-7-ene (DBU) (Route A).[3] Compounds **24**,**35**,**47** and **56** were synthesized by condensation of 5-trichloromethyloxadiazoles (**III**) with anilines (**IV**) (Route B),[4] because the corresponding pure imidocarbonylchlorides were not obtained by the general method. 5-Anilino-thiadiazoles were obtained by condensing amidines (**V**) with phenylisothiacyanates (**VI**) followed by oxidative cyclization with bromine (Fig. 2).[5] Typical procedures are described in the following examples. Identification of the compounds was confirmed by ^{1}H NMR and MS spectroscopy. NMR spectra were measured on a JOEL FX-270 spectrometer with tetramethylsilane as an internal reference. Mass spectra were measured on a Hitachi

[1] Study on new squalene epoxidase inhibitors (Part 1)

* To whom correspondence should be addressed.

Present address: Polymer Laboratory, Advanced Materials Research Center, Kansai Research Institute, Kyoto Research Park, 17 Chudoji Minami-machi, Shimogyo-ku, Kyoto 600-8813, Japan

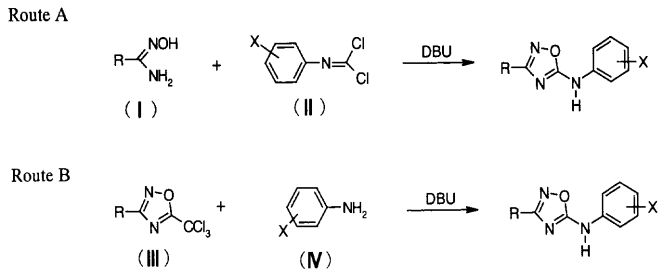


Fig. 1 Synthesis of 5-anilino-oxadiazoles.

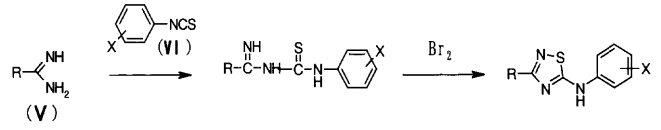


Fig. 2 Synthesis of 5-anilino-thiadiazoles.

M-80 mass spectrometer. Melting points and refractive indexes are uncorrected.

*1.1 Synthesis of 3-(2,6-dichlorophenyl)-5-(4-chlorophenylamino)-1,2,4-oxadiazole (**50**)*

To a solution of 2,6-dichlorobenzamidoxime (5.0 g) and *N*-(*p*-chlorophenyl)imidocarbonylchloride (5.1 g) dissolved in chloroform (100 ml), DBU (3.7 g) in chloroform (20 ml) was added dropwise at 0°C. The mixture was kept at room temperature for 12 hr. Solvent was removed under reduced pressure and the reaction mixture was poured into water and extracted with ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The reaction product was purified by silicagel column chromatography (*n*-hexane: ethyl acetate 9:1) to give **50** (2.3 g, yield 30.8%). mp 195–196°C ^{1}H NMR($CDCl_3$) δ ppm: 7.2–7.45(7H,m,aromatic), 8.75(1H,s,N-H) EI-MS *m/z*:339(M^+), 213, 186(100%), 171, 153, 125.

*1.2 Synthesis of 3-(2,6-dichlorophenyl)-5-(phenylamino)-1,2,4-oxadiazole (**47**)*

DBU(2.3 g) was added dropwise to a solution of 3-(2,6-dichlorophenyl)-5-trichloromethyl-1,2,4-oxadiazole (1.5 g) and aniline (1.4 g) in dimethyl sulfoxide (DMSO) (20 ml) under ice-cooling. The mixture kept at room temperature overnight was poured into ice-water, and extracted with ethyl acetate, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The reaction product was purified by silicagel column chromatography (*n*-hexane: ethyl acetate 9:1) to obtain **47** (0.4 g, yield 30.2%). mp 194–196°C ^{1}H NMR ($CDCl_3$) δ ppm: 7.1–7.48(8H,m, aromatic), 8.35(1H,s,N-H) EI-MS *m/z*: 305(M^+), 213, 186(100%), 171, 153.

*1.3 Synthesis of 3-(2-methylbenzyl)-5-(4-chlorophenylamino)-1,2,4-thiadiazole (**28**)*

A mixture of 2-methylbenzylamidine(1.0 g), 4-chlorophenylisothiocyanate (1.1 g) in *N,N*-dimethylformamide were stirred at room temperature overnight. The mixture was poured into ice-water, and extracted with ethyl ether, dried with anhydrous magnesium sulfate and concentrated under reduced pressure. The reaction product, imidoylthiourea was crystallized with benzene and *n*-hexane. To a solution of imidoylthiourea (0.5 g) in methylene chloride, pyridine (0.12 g) and bromine (0.25 g) were added dropwise under ice-cooling. The mixture was stood for 1.5 hr at room temperature. The mixture was poured into ice-water, extracted with ethyl ether, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The compound was purified by silicagel column chromatography(*n*-hexane: ethyl acetate 9:1) to afford compound **28** (0.45 g, yield 18.8%). mp 133–134°C ^{1}H NMR ($CDCl_3$) δ ppm: 2.35 (3H,s,CH_3), 4.12(2H,s,CH_2), 7.10–7.40(8H,m,aromatic), 7.80(1H,s,N-H) EI-MS *m/z*: 315(M^+,100%), 267, 189, 163, 130, 105.

2. Biological Tests

Fungal strains used in this study were *C. albicans* (IFO 0579), *T. mentagrophytes* (IFO 6202) and *Botrytis cinerea* (Bc-9, supplied by Kanagawa Horticultural Research Station) and *Pyricularia oryzae* (Po-5, isolated in the Biological Laboratory of our research center). Fungicidal activity in Sabouraud's glucose broth medium (glucose: 2%, polypeptone: 1%) or potato dextrose (2%) broth medium was measured by using 96 well microtiterplate (Falcon 35-3072, Becton Dickinson Labware). Test compounds dissolved in DMSO were added to each well where the concentration of DMSO was set to 0.8%. After incubation for 7 days at 30°C (*T. mentagrophytes*, 1×10^5 spores/ml) or 3 days at 20°C (*B. cinerea* and *P. oryzae*, 3×10^4 spores/ml), the growth of mycelium was measured by determining absorbance at 450 nm with a microplate reader (Bio-Tek Instruments Inc. EL-340). The concentration required to give 50% inhibition of growth (EC_{50}) and minimum concentration required to totally inhibit growth (MIC) were calculated graphically. After incubation, conidia treated with test compounds were observed morphologically with a microscope (Olympus IMT-2).

3. Analysis of Sterols

Sterol biosynthesis assay was performed according to the established procedures[6–8] with some modifications. Two ml of cell suspension (2×10^8 cells/ml) of *C. albicans* were inoculated into 100 ml of Sabouraud's glucose broth medium in a 200 ml Erlenmeyer flask, and test compounds dissolved in DMSO were added to the culture and then incubated at 37°C for 21 hr with gentle shaking. After incubation, lipid extraction of harvested cells was carried out with chloroform:methanol (1:2,v/v). The solution was condensed under reduced pressure, and hydrolyzed with 10% KOH in 60% ethanol (v/v) at 80°C for 1 hr, then extracted with hexane twice. Analysis of extracted lipids was performed with a Shimadzu GC-7A

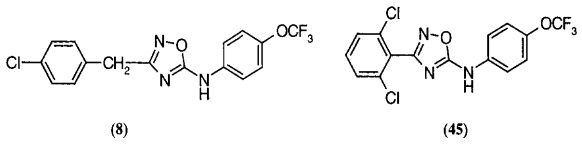
gas-liquid chromatograph (GLC) equipped with a flame-ionization detector. A diasolid column (3 mm×2 m) was used at 280°C, and the carrier gas was nitrogen with a flow rate 20 ml/min. Retention times were compared with authentic standards.

Ten μg of solution of compound in DMSO was added to 1.9 ml of mycelial suspension (*B. cinerea* in potato dextrose broth medium and *T. mentagrophytes* in Sabouraud's glucose broth medium), then 37 kBq [2-^{14}C] acetate (sp.ac. 2,035 MBq/mmol, NEN) was added. Mycelia incubated for 3 hr were saponified with 15% KOH in 60% ethanol (v/v) at 80°C, and extracted with hexane twice. Squalene in the unsaponifiable fraction was separated from other fractions on thin layer chromatogram (TLC plates: Silicagel 60 F_{254} precoated, 0.25 mm thick 20×20 cm, Merck) with *n*-heptane: diisopropyl ether: acetic acid (60:40:4) and the detection of radioactivity on TLC plates was performed with a liquid scintillation spectrometer (Aloka LSC-5100).

RESULTS AND DISCUSSION

1. *Antifungal Activity and Microscopic Observation*

In the screening process we found that two oxadiazole compounds (Fig. 3) showed antifungal activity against *T. mentagrophytes*. Microscopic observation revealed that these compounds inhibited mycelial growth with morphological changes without inhibition of germination as shown in Fig. 4-C. Cultured hyphal tips in the liquid medium were distorted, swollen and excessively branched. The same morphological characterisitic was observed in mycelium treated with clotrimazole, an ergosterol inhibitor (Fig. 4-B). This finding suggested that these compounds disturbed normal membrane formation through the inhibition of sterol biosynthesis. Kato *et al*. have reported similar morphological changes in mycelia of *Monilinia fructigena* when buthiobate, an ergosterol inhibitor, was treated.[6]



(8) (45)

Fig. 3 The first lead compounds.

2. *Analysis of Sterols*

Fig. 5-A and 5-B show GLC profiles of sterols extracted with chloroform-methanol from *C. albicans* cells treated with and without the test compounds, respectively. The major component of sterols in untreated *C. albicans* was ergosterol (Peak 2), and only a little squalene (Peak 1) was observed at retention time of 2.70 min (Fig. 5-A). When treated with compound **8**, ergosterol (Peak 2) decreased strikingly and much squalene (Peak 1) was accumulated (Fig. 5-B). Compound **45** also showed accumulation of squalene (data not shown). These results strongly suggested that the compounds were new squalene epoxidase inhibitors. Consequently we were prompted to synthesize the oxadiazoles and related compounds having various substituents to investigate the

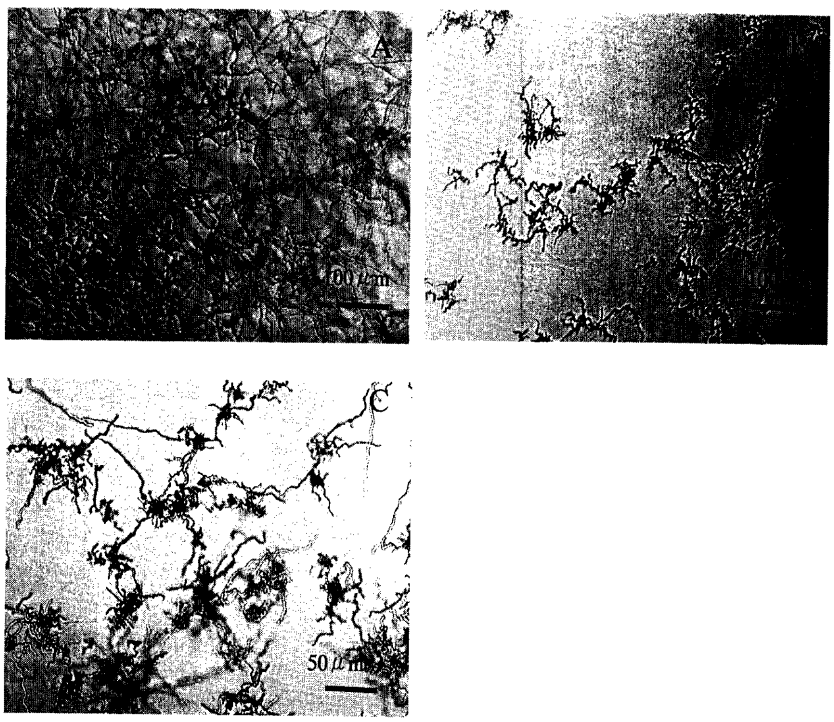


Fig. 4 Morphological Changes of *T. mentagrophytes*.
Conidia in the liquid medium were observed with an optical microscope. A: untreated control, B: clotrimazole 0.03 μg/ml, C: compound **8** 0.1 μg/ml.

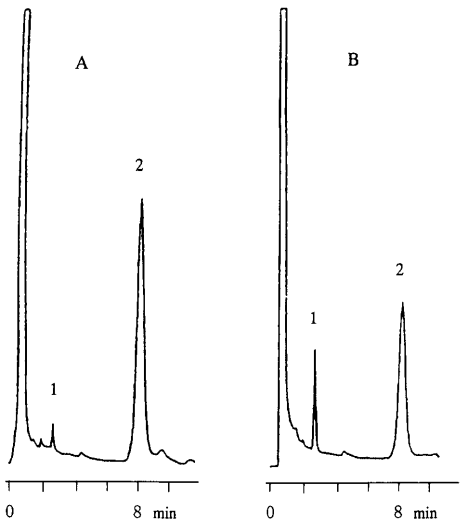


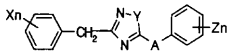
Fig. 5 Gas-liquid chromatograms of sterol components of *C. albicans* cells incubated for 24 hr without and with compound **8**.
A: untreated, B: treated with compound **8** 5 μg/ml. Peak 1: squalene (Retention time 2.70 min), Peak 2: ergosterol (Retention time 8.14 min).

structure-activity relationships.

3. *Fungicidal Activities of 3-Benzyl-1,2,4-Oxadiazoles and 1,2,4-Thiadiazoles*

Table 1 shows antifungal activities of substituted 3-benzyl-5-anilino-oxadiazoles and -thiadiazoles against *T. mentagrophytes, B. cinerea* and *P. oryzae*, among which *T. mentagrophytes* was most sensitive to the chemicals. All the compounds except that with 2-phenyl (**5**) showed moderate or high activities, when the substituent on anilino group was fixed to *para* chloro (**1**–**7**, **9**–**16**). The 3-benzyloxadiazoles showed moderate activities against *P.oryzae*, but they were not or less active against *B. cinerea*. Among 3-naphthylmethyloxadiazoles (**17**, **18**), the α-naphthylmethyl compound **17** showed higher activity than the β-analogue **18** against *T. mentagrophytes*, indicating that a steric effect of the arylmethy group plays an important role for the activity. Substituent effects of 5-anilino moiety were examined for a series of oxadiazoles having 2-methylbenzyl group at the 3-position. Among chloro-substituted anilino compounds, the 4-

Table 1 Structures, physical properties and fungicidal activities of 3-benzyl-5-anilino-1,2,4-oxadiazoles and 1,2,4-thiadiazoles.



Compound No.	Xn	Y	Zn	A	mp (℃) or n_D	MIC[a] *T.m.*	EC_{50}[a] *B.c.*	EC_{50}[a] *P.o.*
1	H	O	4-Cl	NH	154-155	1	>100	3
2	2-CH_3	O	4-Cl	NH	83-84	0.3	>100	0.6
3	2-Cl	O	4-Cl	NH	152-153	0.3	>100	1
4	2-OCH_3	O	4-Cl	NH	132-133	1	>100	>100
5	2-C_6H_5	O	4-Cl	NH	n_D^{24} 1.5915	100	>100	1
6	3-CH_3	O	4-Cl	NH	144-145	1	>100	0.6
7	4-CH_3	O	4-Cl	NH	149	1	>100	(0.6)[b]
8	4-Cl	O	4-OCF_3	NH	107-108	3	>100	0.6
9	2,4-Cl_2	O	4-Cl	NH	134-135	3	>100	0.6
10	3,4-Cl_2	O	4-Cl	NH	143-144	3	(30)[b]	0.6
11	2,3-$(CH_3)_2$	O	4-Cl	NH	152-153	0.3	>100	0.6
12	2,4-$(CH_3)_2$	O	4-Cl	NH	109-111	0.1	20	0.3
13	2,5-$(CH_3)_2$	O	4-Cl	NH	124-125	0.1	100	1
14	2,6-$(CH_3)_2$	O	4-Cl	NH	165-166	100	>100	>100
15	3,4-$(CH_3)_2$	O	4-Cl	NH	106-107	0.3	>100	1
16	3,5-$(CH_3)_2$	O	4-Cl	NH	147-148	0.3	>100	1
17	2,3-(CH=CH)$_2$-	O	4-Cl	NH	181-182	(1)[b]	>100	>100
18	3,4-(CH=CH)$_2$-	O	4-Cl	NH	200-201	>100	>100	>100
19	2-CH_3	O	2-Cl	NH	86-87	>100	>100	3
20	2-CH_3	O	3-Cl	NH	78-81	1	>100	1
21	2-CH_3	S	3-Cl	NH	109-111	(10)[b]	(30)[b]	1
22	2-CH_3	S	3-CF_3	NH	129-130	>100	>100	1
23	2-CH_3	O	4-CH_3	NH	117-118	0.1	>100	2
24	2-CH_3	O	4-C_4H_9t	NH	145-146	(3)	>100	2
25	2-CH_3	S	4-C_4H_9t	NH	145-147	>100	>100	>100
26	2-CH_3	O	4-OCH_3	NH	89-91	0.3	10	1
27	2-CH_3	O	4-F	NH	118-119	1	>100	3
28	2-CH_3	S	4-Cl	NH	133-134	1	(20)[b]	0.3
29	2-CH_3	S	4-Br	NH	147-149	1	>100	0.3
30	2-CH_3	O	4-CF_3	NH	115-116	0.3	>100	0.3
31	2-CH_3	S	4-OCF_3	NH	117-118	3	>100	0.6
32	2-CH_3	O	3,4-Cl_2	NH	83-84	0.3	>100	0.3
33	2-CH_3	O	4-Cl	N(CH_3)	n_D^{25} 1.5995	>100	>100	3
34	2-CH_3	O	4-Cl	CH_2	n_D^{25} 1.5763	>100	>100	6

[a] MIC,EC_{50} : μg/ml [b] caluculated by extrapolation because of low water solubility

chloro derivative (**2**) exhibited the strongest activity, while the 3-chloro derivative (**20**) was moderately active and the 2-chloro analog (**19**) showed no activity against *T. mentagrophytes*. In general, 4-substituted analogs showed high activities against *T. mentagrophytes* and *P. oryzae*. Thiadiazole compounds exhibited equivalent or rather lower fungicidal activities compared to the corresponding oxadiazoles (**20** vs. **21**, **24** vs. **25**, **2** vs. **28**). The *N*-methylanilino analog (**33**) and the 5-benzyl analog (**34**) were inactive against *T. mentagrophytes*.

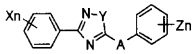
4. *Fungicidal Activities of 3-Phenyl-1,2,4-Oxadiazoles and 1,2,4-Thiadiazoles*

Table 2 shows fungicidal activity of 3-phenyl-5-anilino-oxadiazoles and -thiadiazoles. The substituent effects of the 3-phenyl group to the activities were assessed in 4-chloro- or 4-trifluoromethoxy-anilino derivatives (**36**-**46**). Only the 2,6-dichloro analogs (**45**,**46**) maintained high fungicidal activities, but other derivatives (**36**-**44**) were completely inactive at 100 μg/ml. In the 2,6-dichlorophenyl derivatives, introduction of an electron-withdrawing lipophilic substituent at *para*-position of the anilino group gave high fungicidal activities (**45**,**46**,**50**,**57**). Thiadiazole compounds were more active than the corresponding oxadiazoles (**51** vs. **46**, **58** vs. **57**, **59** vs. **45**). In this series 3-(2,6-dichlorophenyl)-5-(4-chloroanilino) (**51**) and -(4-bromoanilino) thiadiazoles (**52**) showed highest fungicidal activities against *T. mentagrophytes, B. cinerea* and *P. oryzae*. Replacement of the 5-anilino group by *N*-methyl anilino (**62**) or benzyl group (**63**) remarkably decreased the fungicidal activity against *T.mentagrophytes* as similar as it was found in the 3-benzyl derivatives (**33**,**34**).

5. *Accumulation of Squalene*

Table 3 shows accumulation of squalene synthesized from [2-^{14}C]-acetic acid in *B. cinerea* and *T. mentagrophytes* after treatment of compounds **2**, **28**, **50** and **51**. Although compound **50** did not show any activity against *B. cinerea*, accumulation of squalene was significantly observed at 1×10^{-5} M. Benzyl oxadiazole (**2**) and benzyl thiadiazole (**28**) accumulated squalene slightly at 5×10^{-5} M in *B. cinerea*, which is compatible with their weak fungicidal activity against B. cinerea. In sensitive fungi, *T. mentagrophytes*, accumulation of squalene was observed remarkably at 1×10^{-7} M. Compound **51** showed as potent fungicidal activity as tolnaftate and naftifine in *T. mentagrophytes* and *B. ciner-*

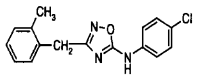
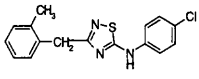
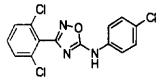
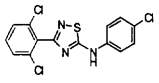
Table 2 Structures, physical properties and fungicidal activities of 3-phenyl-5-anilino-1,2,4- oxadiazoles and 1,2,4-thiadiazoles.



Compound No.	Xn	Y	Zn	A	mp (℃) or n_D	MIC[a] *T.m.*	EC_{50}[a] *B.c.*	EC_{50}[a] *P.o.*
35	H	O	H	NH	140-141	>100	>100	>100
36	H	O	4-OCF_3	NH	194-195	>100	>100	>100
37	4-Cl	O	4-OCF_3	NH	187-190	>100	>100	>100
38	4-CH_3	O	4-OCF_3	NH	212-217	>100	>100	>100
39	3-Cl	O	4-OCF_3	NH	167-171	>100	>100	>100
40	2-Cl	O	4-Cl	NH	210	>100	>100	>100
41	2,6-F_2	O	4-Cl	NH	205-206	>100	>100	>100
42	2,6-$(CH_3)_2$	O	4-Cl	NH	192-193	>100	>100	>100
43	2,3-$(CH{=}CH)_2$-	O	4-Cl	NH	205-206	>100	>100	>100
44	3,4-$(CH{=}CH)_2$-	O	4-Cl	NH	214-215	>100	>100	>100
45	2,6-Cl_2	O	4-OCF_3	NH	169-170	1	>100	0.6
46	2,6-Cl_2	O	4-Cl	NH	195-196	3	>100	0.6
47	2,6-Cl_2	O	H	NH	194-196	(100)[b]	>100	>100
48	2,6-Cl_2	O	2-Cl	NH	180-183	>100	>100	>100
49	2,6-Cl_2	O	3-Cl	NH	176-177	>100	>100	0.5
50	2,6-Cl_2	O	4-Cl	NH	195-196	3	>100	0.6
51	2,6-Cl_2	S	4-Cl	NH	214-218	0.1	0.2	0.6
52	2,6-Cl_2	S	4-Br	NH	205-206	0.1	0.2	0.4
53	2,6-Cl_2	O	4-F	NH	219-220	>100	>100	>100
54	2,6-Cl_2	O	4-OCH_3	NH	186-188	(100)[b]	>100	>100
55	2,6-Cl_2	O	4-CH_3	NH	179-181	(10-30)[b]	>100	>100
56	2,6-Cl_2	O	4-C_4H_9t	NH	191-192	>100	>100	>100
57	2,6-Cl_2	O	4-CF_3	NH	169-170	1	>100	0.6
58	2,6-Cl_2	S	4-CF_3	NH	164-166	0.3	1	0.2
59	2,6-Cl_2	S	4-OCF_3	NH	188-190	0.3	0.6	0.3
60	2,6-Cl_2	O	2,3-$(CH{=}CH)_2$-	NH	234-235	(100)[b]	>100	>100
61	2,6-Cl_2	O	3,4-Cl_2	NH	218-220	>100	6	0.6
62	2,6-Cl_2	S	4-Cl	$N(CH_3)$	163-164	(10)	>100	1
63	2,6-Cl_2	O	4-Cl	CH_2	93-95	>100	>100	3

[a] MIC,EC_{50} : μg/ml [b] caluculated by extrapolation because of low water solubility

Table 3 Inhibition of squalene epoxidase by the oxadiazoles and the thiadiazoles in *T. mentagrophytes* and *B. cinerea*.

Compound No.	Structure	*T. mentagrophytes* MIC (μg/ml)	Accumulation of Squalene (%) 1×10^{-9}M	1×10^{-8}M	1×10^{-7}M	1×10^{-6}M	*B. cinerea* EC_{50} (μg/ml)	Accumulation of Squalene (%) 1×10^{-7}M	1×10^{-6}M	1×10^{-5}M	5×10^{-5}M
2		0.3		4.7	49.9	71.3	>100			1.8	12.0
28		1		7.5	35.9	73.3	(20)			3.8	43.0
50		3		4.1	46.8	66.5	>100		4.2	52.5	68.6
51		0.1		42.8	75.2	76.5	0.2	9.4	64.4	82.0	
	tolnaftate[a)]	0.1	35	72.1	81.5	81.4	0.1	10.0	66.0	79.0	
	naftifine[a)]	0.03	20	63.4	81.9	78.9	0.6		1.7	55.0	

Cells were incubated with ^{14}C-acetate in the presence of compounds.
Accumulation of squalene was caluculated with equation A(%)= (squalene/ total unsaponifiable lipids) × 100.
In the absence of the compounds, 2.7% and 1.6% squalene was accumulated in *T. mentagrophytes* and *B. cinerea*, respectively.
a) See Fig.6-A,B

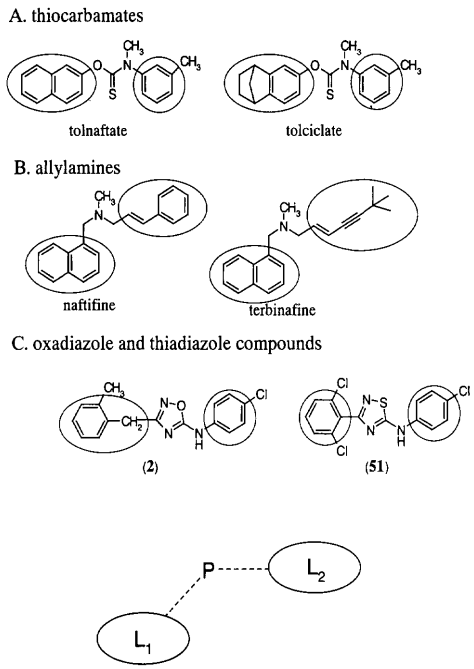


Fig. 6 Structures of miscellaneous squalene epoxidase inhibitors (A,B,C) and its general structural feature.[10]
L_1, L_2: Lipophilic domains; P: a polar center. Lipophilic domains corresponding to L_1 or L_2 are circled.

ea, and 42.8% and 64.4% squalene was accumulated when they were treated with **51** at 1×10^{-8} M and 1×10^{-6} M, respectively.

Two classes of synthetic squalene epoxidase inhibitors are known, *i.e.* thiocarbamates (such as tolnaftate and tolciclate) and allylamines (such as naftifine and terbinafine) (Fig. 6-A,B).[9] Ryder has briefly pointed out that the thiocarbamates have some structural similarity to the allylamines in terms of overall molecular shape.[9] Nussbaumer *et al*. proposed a pharmacophoric model of squalene epoxidase inhibitors, in which two lipophilic domains are linked by a spacer of appropriate length containing a polar center as shown in Fig. 6.[10] In this model, at least one of the lipophilic domains represents bicyclic aromatic ring system (*e.g.* naphthalene or tetrahydronaphthalene). Although the oxadiazole and thiadiazole compounds in the present study contain no naphthalene moiety, they have two lipophilic domains, benzyl or 2,6-dichlorophenyl group and substituted anilino group (Fig. 6-C). The oxadiazole or thiadiazole moiety corresponds to the spacer with a polar center according to the Nussbaumer's model. Among the compounds investigated in this paper, 3-(2,6-dichlorophenyl)-5-(4-chloroanilino)thiadiazole (**51**) showed the highest fungicidal activity, and accumulated squalene remarkably as shown above. This compound is of interest as a new lead of squalene epoxidase inhibitors, and further synthetic and biological studies are hopefully expected to develop a new class of practical inhibitors in the third generation after the thiocarbamates and the allylamines.

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要　　約

抗菌活性を有する5-アニリノ-1,2,4-オキサジアゾールと5-アニリノ-1,2,4-チアジアゾールの合成およびそのステロール生合成阻害活性

汲田　泉，丹羽　淳

種々の目的で合成された化合物ライブラリーについて *in vitro* 抗菌試験を行い，1,2,4-オキサジアゾール誘導体の2化合物が *T. mentagrophytes* に対し比較的高い抗菌活性を示すことを見出した．これらの化合物は顕微鏡観察において菌糸の縮れた特徴的な形態変化を伴う生育抑制を示した．さらに，薬剤を処理した *Candida* 菌体のステロールの分析をガスクロマトグラフィーにより行うと，スクワレンの顕著な蓄積がみられ，スクワレンエポキシダーゼを阻害しているものと思われた．これらの化合物をリード化合物として合成展開を図り，3-(2,6-ジクロルフェニル)-5-フェニルアミノ-1,2,4-チアジアゾール類を見出した．これらの化合物は *T. mentagrophytes* や *B. cinerera* に対して高い抗菌活性を示すと同時に，それに対応した強いスクワレンエポキシダーゼ阻害活性を示した．新しく見出されたチアジアゾール誘導体は第3のタイプのスクワレンエポキシダーゼ阻害剤であり，リード化合物としてさらに興味がもたれる．